ORIGINAL ARTICLE

Factors related to the chronicity and evolution of hepatitis C infection in patients co-infected by the human immunodeficiency virus

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Objectives This work analyses the influence of immune status, serum human immunodeficiency virus (HIV) load and hepatitis C virus (HCV) genotypes on the probability of resolution of HCV infection in HIV-co-infected patients, as well as the evolution of HCV viremia after antiretroviral therapy.

Patients and methods Forty-five patients with anti-HIV and anti-HCV antibodies were classified into two groups as a function of the positivity or persistent negativity of HCV RNA detection (active or recovered HCV infection, respectively). They were treated with highly active antiretroviral therapy (HAART). Serum HCV RNA was quantified by the reverse transcription-polymerase chain reaction. HCV genotypes were detected by line probe assay or by detection of type-specific antibodies.

Results HCV RNA was detectable in 30 (66.6%) out of 45 HIV-infected patients. $CD4^+$ T-cell counts, HIV viremia, or HCV genotypes were similar in patients with active or recovered HCV infection. Patients with active HCV infection had a non-significant decrease of HCV viremia during a follow-up of 12 months (from 6.15 ± 6.32 to 5.96 ± 6.05 log copies/mL). This was not influenced by baseline HCV or HIV viral load, HCV genotype, or $CD4^+$ T-cell count. The non-significant decrease was present in patients with or without an immunological response to HAART.

Conclusion HCV genotypes, immune status, or serum HIV load did not influence the resolution or chronicity of HCV infection in HIV-co-infected individuals. A non-significant decrease of HCV viremia in these patients treated with combinations including antiproteases could be expected.

Keywords Hepatitis C virus, human immunodeficiency virus, chronicity, hepatitis C virus genotypes

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INTRODUCTION

Hepatitis C virus (HCV) infection is becoming an important cause of morbidity and mortality in patients co-infected with human immunodeficiency virus (HIV) [1,2]. Cross-sectional studies have detected higher HCV viremia levels in patients co-infected by HIV [3–6]. Likewise, it has been demonstrated that HIV infection modifies the natural course of chronic HCV disease, producing an unusually rapid progression to cirrhosis [7].

Controversial results about the influence of the level of immunodepression on the evolution of HCV infection have been published [8,9]. One criticism of these studies is that most did not include genotype analysis on HCV. Several articles have suggested an accelerated progression

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towards cirrhosis in non-HIV-infected patients with chronic HCV infection due to genotypes 1a and 1b [10–13].

Finally, the effects of the treatment with potent antiretroviral agents on the course of HCV infection have recently been addressed: stabilization of HCV viremia has been demonstrated during combination therapy including antiproteases [14–16], although the existing data are controversial [17]. The influence of infection by specific HCV genotypes has not been analysed in this population.

All the foregoing information concerns the evolution towards advanced stages of liver disease in patients with chronic HCV infection. Studies of those parameters implicated in resolution or chronicity after HCV seroconversion are scarce, both in HIV-infected and in HIV-non-infected individuals [18]. The lack of a clinically significant episode of acute infection in most HCV-infected patients could explain the absence of this information [19].

In the present study we analyse the putative factors associated with HCV chronicity in a cohort of HIV-co-infected patients. Also, the influence of implicated HCV genotypes, baseline HCV viral load, level of immunodepression and response to antiretroviral agents on the evolution of HCV viremia are considered.

PATIENTS AND METHODS

We carried out a prospective study of 45 patients in the Hospital Universitario Puerta del Mar (Cádiz, Spain). This tertiary center serves a population of 270 000 inhabitants. Cumulative prevalence of acquired immunodeficiency syndrome (AIDS) in our area is 660 per million inhabitants.

Study population

Patients were consecutively selected from outpatients who attended the Infectious Disease Unit, on the basis of simultaneous positivity for anti-HIV and anti-HCV antibodies. Each one was asymptomatic with respect to HIV infection and liver disease, and the echographic study showed no evidence of portal hypertension.

Patients were classified into two groups on the basis of the positivity or persistent negativity of HCV RNA detection (active or recovered HCV infection, respectively). A factor complicating the analysis of chronicity of HCV infection is the possibility of intermittent positivity of serum HCV RNA determination [20,21]. Thus, a stringent criterion, negative results in each of three samples taken at 3-month intervals, was required to define an individual as an HCV non-viremic patient (recovered HCV infection). Patients with serum HCV RNA positivity in at least one determination are referred to as HCV viremic patients (active HCV infection). The characteristics of the study population are presented in Table 1.

In accordance with previous articles, the duration of HCV infection was estimated as the interval in years from blood transfusion or, in intravenous drug users, the first sharing of needles [3,22].

Exclusion criteria were: (1) previous anti-HIV or anti-HCV treatments; (2) immunomodulatory therapy (corticoids, immunosuppressors, pentoxyfilline) that could modify the evolution of the infection; (3) presence of opportunistic diseases related to HIV infection [23]; (4) active co-infection by other hepatotropic viruses (active infection by hepatitis virus B or delta, cytomegalovirus, or Epstein–Barr virus was absent); (5) evidence of other toxic (ethanol consumption, drugs), metabolic (serum levels of α1-antitrypsin, ceruloplasmin and iron were normal), or immunological (anti-nuclear, anti-smooth muscle and anti-liver kidney microsomal antibodies were negative) causes of liver disease; and (6) because of the possibility of HCV superinfections, active drug users were excluded.

The study protocol was approved by the Institutional Ethical Committee and all patients and controls gave their informed consent.

Laboratory procedures

Sera reactivity for anti-HIV was performed by enzyme immunoassay (EIA; Abbott Laboratories, North Chicago, IL, USA) and confirmed by Western blot (Pasteur Institute, Paris, France). Serum HIV load was determined by reverse transcription–polymerase chain reaction (RT-PCRl Amplicor HIV, Roche Diagnostics, Basel, Switzerland). The sensitivity of the assay is 400 RNA copies per ml of serum.

Sera of all subjects were reactive for anti-HCV, according to both a second-generation enzyme immunoassay (EIA-2; Ortho Diagnostic System, Raritan, NJ, USA) and a second-generation recom-

Table 1 Characteristics of the study population

Parameter	HCV-RNA-positive patients ($n = 30$)	HCV-RNA-negative patients ($n = 15$)
Age (years)	31.3 ± 6.1	31.1 ± 5.0
Male : female ratio	25 : 5	11 : 4
Risk factors for HIV infection (*n*,%)		
Drug use	29 (96.7)	12 (80.0)
Haemoderivative recipients	1 (3.3)	0 (0)
Heterosexuality	0 (0)	3 (20.0)*
Time from the onset of risk conduct (months)	69.6 ± 42.8	73.1 ± 41.6
Previous opportunistic diseases	4 (13.2)	3 (20.0)
Serum HIV load (log copies/mL)	4.89 ± 5.19	4.69 ± 5.19
$CD4^+$ T-cell count/mm^3	515.0 ± 301.8	483.1 ± 268.9
Genotype/serotype		
1 a	16 (53.3)	10 (66.7)
1 b	7 (23.3)	
1 a/1 b	7 (23.3)	
1?	1 (3.3)	
3	10 (33.3)	2 (13.3)
3 a	9 (30.0)	
3 b	1 (3.3)	
4	4 (13.4)	2 (13.3)
4 c/4 d	4 (13.4)	
Multiple types	0 (0)	1 (6.6) †

*$P = 0.032$. †Serotypes 1 and 3.

binant immunoblot assay (RIBA-2; Ortho Diagnostic System). Serum samples were tested for HCV RNA using the RT-PCR (Amplicor Monitor HCV Assay, Roche Diagnostics), with primers derived from the highly conserved 5′-untranslated region, as previously described [24]. The sensitivity of the assay is 1000 RNA copies per ml of serum.

In those patients with serum positivity for HCV RNA, isolates were genotyped by line probe assay (INNO-LiPA HCV; Innogenetics, Antwerp, Belgium), as previously described [25]. The HCV genotype nomenclature used in this report is that proposed by an international panel [26].

In patients whose sera were negative for HCV RNA and in a random sample of 15 patients whose sera were positive for HCV RNA, antibodies specific for genotypes implicated in HCV infection were detected by the Murex HCV serotyping 1–6 assay, as previously described [27]. This assay aims to detect the type-specific antibodies against non-structural region 4-derived epitopes. Serotyping was performed according to the manufacturer's instructions. The results of this approach represent serologically defined genotypes. However, to facilitate their differentiation from genotypes defined by line probe assay, they will be designated as serotypes in this article.

Study schedule

Each patient was treated with zidovudine (200 mg/8 h), zalcitabine (0.75 mg/8 h) and saquinavir (600 mg/8 h), as well as with prophylaxis against opportunistic diseases, according to a prospective protocol [28,29]. They did not receive treatment against HCV infection. Evaluations were performed at 3, 6 and 12 months. Clinical status, haemogram, serum biochemistry, $CD4^+$ T-cell counts, serum HIV load and, in active HCV infection, HCV load were analysed in each of these evaluations. HCV genotyping and serotyping analyses were performed at the beginning of the study.

An immunological response to the antiretroviral regimen was considered when $CD4^+$ T-lympho-

cyte counts increased by more than 25% above baseline levels. A virological response was considered when the HIV load decreased by more than 1 log copy/mL after 12 months of therapy.

Statistical analysis

Data are presented as mean ± standard deviation or, when indicated, as absolute number and percentage. The data from two independent groups were compared with the Mann–Whitney *U*-test. Significance of parameters within each group was measured by the Wilcoxon matched-pairs signed rank test. For qualitative variables, χ^2 with Yates' correction or Fisher's exact test was used.

The relation of co-variates (age, sex, risk factors for HCV–HIV infection, time from the onset of risk conduct, $CD4^+$ T-cell counts, serum HIV load and HCV types) with HCV viremia (dependent variable) was assessed by univariate analysis and the odds ratios (O.R.), and confidence intervals at 95% (CI 95%) were determined. A *P*-value of <0.05 at χ^2 was considered statistically significant.

RESULTS

HCV RNA was detectable in 30 (66.6%) out of 45 HIV-infected patients. Serum transaminase concentrations were higher in HCV viremic patients aspartate aminotransferase (AST): 66.4 ± 49.1 IU/L, range 15–243 vs. 28.5 ± 22.6 IU/L, range 13–42; alanine aminotransferase (ALT): 87.2 ± 79.2 IU/L, range 9–260 vs. 27.3 ± 24.5 IU/L, range 7–41; $P < 0.001$ in each case). However, nine (30%) of the 30 HCV viremic patients presented with normal serum levels of AST and ALT. No HCV-RNA-negative patient showed elevation of transaminases above the normal range.

Results of genotyping and serotyping performed in patients with active or past HCV infection are presented in Table 1. Although a higher prevalence of HCV type 3 and a lower prevalence of HCV type 1 was observed in HCV-RNA-positive patients, this difference did not reach statistical significance with HCV-RNA-negative patients. Concordance between serotyping and genotyping methods was analysed in a sample of 15 HCV viremic patients (genotype 1 a, 1 b and 3 a, four cases each; genotype 4 c/4 d, three cases). In each patient, type-specific antibodies identified a serotype that was concordant with the genotype. This includes three samples (20%) with a single genotype (3 a) but a double serotype (1 + 3).

With the objective of quantifying the relationship between the several analysed co-variates and HCV viremia, demographic factors, HIV-related parameters and HCV types, considered as independent variables were correlated with the presence or absence of serum HCV RNA (dependent variable) by univariate analysis. No statistically significant association was detected (Table 2).

In HCV-RNA-positive patients, the mean HCV viremia was 6.15 ± 6.32 log copies/mL. A signifi-

Table 2 Univariant analysis of putative factors implicated in chronicity of HCV infection in HIV-co-infected patients

Parameter	Values	HCV-RNA positive/ HCV-RNA negative	Odds ratio	Confidence interval 95%	*P*
Age	>35 years	20/13	0.31	0.03–1.81	0.283
	<35 years	10/12			
Gender	Male	25/11	1.82	0.30–10.25	0.454
	Female	5/4			
Time from acquisition of disease	>5 years	19/8	1.51	0.36–6.39	0.747
	<5 years	11/7			
HVC type	1	16/10	0.57	0.12–2.43	0.594
	3	10/2	3.25	0.54–34.42	0.283
	4	4/2	1.00	0.12–12.42	1.000
	Multiple types	0/1	0.00	0.00–19.50	0.333
Serum HIV load	>6 log copies/mL	7/1	4.26	0.45–205.51	0.236
	<6 log copies/mL	23/14			
$CD4^+$ T cells/mm^3	<500	13/9	0.51	0.12–2.13	0.461
	>500	17/6			

Table 3 Immune and virological response of HIV patients to antiretroviral treatment

	At start of treatment	At 12th month	Mean increase (%)
Inmune response to antiretroviral therapy ($CD4^+$ cells/mm^3)			
responders (8 patients, 26.7%)	319.5 ± 194.8	521.0 ± 324.7	67.6 ± 45.9
non-responders (22 patients, 73.3%)	586.1 ± 305.4	543.3 ± 313.1	$-9.5 \pm 8.0^*$
Virological response to antiretroviral therapy (log copies/mL)			
responders (12 patients, 40%)	4.72 ± 5.03	3.41 ± 3.60	-38.4 ± 39.7
non-responders (18 patients, 60%)	5.12 ± 5.22	4.89 ± 5.12	$-4.7 \pm 1.9^{**}$

$^*P < 0.001$; $^{**}P = 0.020$.

cantly higher HCV viral load ($P < 0.05$) was detected in patients with infection by HCV type 1 a or 1 b than in those infected by types 3 or 4 (type 1 a: 6.43 ± 6.46 log copies/mL; type 1 b: 6.26 ± 6.26 log copies/mL; type 3 a: 5.67 ± 5.90 log copies/mL; type 4 c/4 d: 5.23 ± 4.88 log copies/mL). Also, higher viral loads were detected in HCV-RNA-positive patients with a longer duration of infection (<5 years ($n = 11$): 5.85 ± 6.20 log copies/mL; 5–10 years ($n = 14$): 6.15 ± 6.26 log copies/mL; >10 years ($n = 5$): 6.46 ± 6.52 log copies/mL; time <5 years vs. time >10 years, $P = 0.020$; other comparisons, $P > 0.05$).

A significant correlation between HCV viral load and the $CD4^+$ T-cell count was not detected ($r = -0.24$, $P > 0.05$). HCV viremia was lower in patients with more than 500 $CD4^+$ T lymphocytes/mm^3 when compared to those with less than 500 $CD4^+$ T lymphocytes/mm^3 (5.87 ± 6.04 vs. 6.28 ± 6.41, $P > 0.05$), although a significant difference was not reached.

Patients received antiretroviral therapy, including an antiprotease, and were followed up for 12 months. At the end of follow-up, a persistent immune response, defined as an increase in $CD4^+$ T-cell counts at the 12th month of more than 25%, was observed in eight (26.7%) patients and a persistent virological response, defined as a decrease of HIV load of more than 1 log copies/mL, was observed in 12 (40.0%) patients (Table 3).

Evolution of serum HCV load and transaminases is presented in Table 4. In HCV-RNA-positive patients, no significant difference in serum transaminases or HCV viral load was detected between the points of evolution considered. Also, in HCV-RNA-negative patients, similar values of serum AST and ALT were detected at every one of these points (Table 4).

With the objective of evaluating the significance of specific HCV genotypes, basal serum HCV and HIV loads, level of immunodepression, and response or absence of response to antiretroviral therapy, patients were grouped into several mutually exclusive categories. Evolution of HCV viral load as a function of these variables is presented in Figures 1 and 2. For every variable considered, no significant difference was detected between the points of evolution considered.

Table 4 Evolution of serum HCV load and transaminases in HIV-infected patients

Group	Parameter	Month 0	Month 3	Month 6	Month 12
HVC-RNA-positive	HCV load (log copies/mL)	6.15 ± 6.32	6.08 ± 6.23	5.97 ± 6.12	5.96 ± 6.05
($n = 30$)	AST (U/L)	66.4 ± 49.1	68.0 ± 35.8	72.2 ± 48.6	63.5 ± 35.0
	ALT (U/L)	87.2 ± 79.2	98.8 ± 76.1	94.3 ± 73.6	91.1 ± 69.7
HVC-RNA-negative					
($n = 15$)	AST (U/L)	28.5 ± 22.6	26.4 ± 22.9	27.3 ± 21.2	18.0 ± 13.5
	ALT (U/L)	27.3 ± 24.5	27.8 ± 39.8	31.1 ± 38.4	14.0 ± 5.3

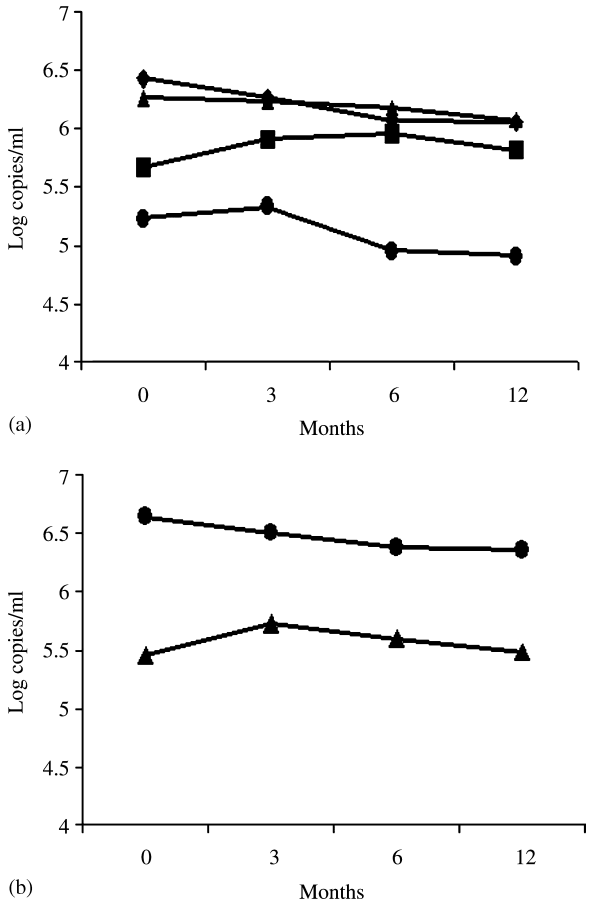

Figure 1 Evolution of mean HCV viremia at 3, 6 and 12 months. (a) Hepatitis C genotype 1a ($n=7$) (◆), genotype 1b ($n=7$) (▲), genotype 3a ($n=9$) (■) and genotype 4c/4d ($n=4$) (●). (b) Baseline HCV viremia: >6 log copies/mL ($n=8$) (●), <6 log copies/mL ($n=22$) (▲).

DISCUSSION

The rate of HCV viremia (serum HCV RNA positive) among those with evidence of past HCV contact (presence of serum anti-HCV antibodies) was 66.6% in our series, a percentage similar to that of other series analysing the rates of chronicity in HIV-non-infected patients [19]. We evaluated the role of demographics, HCV genotypes, immune status, or serum HIV load on the possibility of chronicity or resolution of HCV infection. None of the factors analysed was associated with the chronicity of HCV infection.

The importance of HCV genotype has been addressed in several studies. The absence of a relationship between genotype and evolution is concordant with the evolution of most RNA viruses; for example, different serotypes of dengue virus, another flavivirus, show similar propensities to cause viral haemorrhagic fever [30].

Our results do not support a positive association between either serum HIV load or a more profound immunodepression and chronicity of HCV infection. This was an expected finding [18]. It is assumed that HCV and HIV infections occur simultaneously at the time of blood-derivative transfusion or, in intravenous drug users, in the first year after the onset of drug abuse [3,20]. A profound immunodepression in these first stages of HIV infection is not expected and rates of resolution of HCV infection in HIV-infected patients would be similar to those detected in non-HIV-infected individuals.

Due to the absence of a positive association between the analysed factors and evolution to chronicity, alternative explanations must be sought. Genetic factors [31] and the infecting HCV load could be determining factors that explain the chronicity of the infection.

Both HCV-RNA-positive and -negative patients were followed up for 12 months. The evolution of HCV viremia in HIV-infected patients treated with protease inhibitors has been recently addressed. In the majority of studies, it has been found that HCV viremia remains without significant changes for periods ranging from 8 weeks to 9 months in patients receiving two nucleoside analogues and either Indinavir, Ritonavir, or Saquinavir [14–17], although an elevation of $CD4^+$ T-cell counts and a decrease of HIV viral load occurred [32]. In agreement with these studies, our work has demonstrated a non-significant decrease of HCV viral load. Moreover, we have demonstrated that infecting HCV genotype, time of evolution of the HCV infection, baseline HCV viral load or level of immunodepression have no influence on the evolution of HCV viremia; these parameters have been considered as prognostic factors of chronic HCV infection in non-HIV-infected patients [33].

The absence of modifications of HCV viremia in HIV-infected patients treated with combinations including antiproteases contrasts with the tendency towards increases of HCV viremia found in those patients not receiving anti-HIV treatment [19,33] or receiving only therapy with two nucleoside analogues [34]. There are two possible explanations for this discrepancy. First, immune reconstitution could be responsible for the control

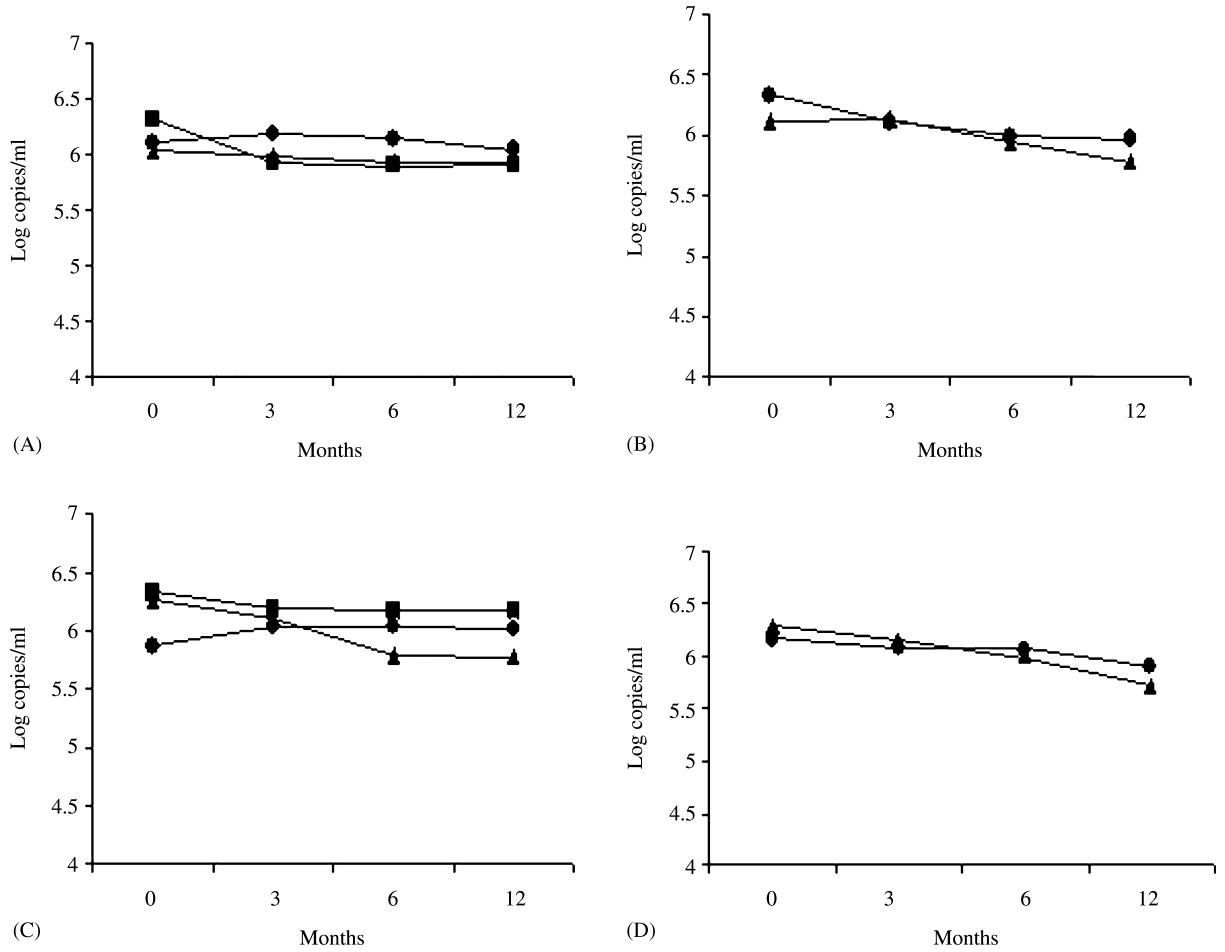

Figure 2 Evolution of mean HCV viremia at 3, 6 and 12 months. (a) Baseline HIV viremia: <3.7 log copies/mL ($n=6$) (●), 3.7–5 log copies/mL ($n=16$) (▲), >5 log copies/mL ($n=8$) (■). (b) Baseline $CD4^+$ T-cell counts/mm^3: >500 ($n=13$) (●), 200–500 ($n=13$) (▲), <200 ($n=4$) (■). (c) Immune response (increase >25% of baseline $CD4^+$ T-cell count) after 12 months of therapy: responders ($n=8$) (●), non-responders ($n=22$) (▲). (d) Virological response (decrease >1 log copies/mL of baseline HIV load) after 12 months of therapy: responders ($n=12$) (●), non-responders ($n=18$) (■).

of HCV viral replication. The absence of differences between patients with immunological response and those without response, detected in the present study, argues against this hypothesis. Second, anti-HIV protease drugs might interact with the recently identified HCV protease [35]. Although this is a possibility, it is evident that this direct effect on HCV replication is suboptimal: HCV viremia remained without significant changes, and did not disappear, in our HIV-infected patients.

Apart from the importance of chronic liver disease as a cause of morbidity and mortality, HCV co-infection has been associated with an accelerated progression of HIV infection and an increased hepatotoxicity due to antiretroviral drugs [36]. Because control of HCV viremia does not coincide with the antiretroviral therapy-induced control of HIV, there is a need for separate treatment of this chronic HCV infection in selected patients.

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